Attorney's Docket No.: 07148-

032001 / CGL99/0007US5A

Applicant: Lorin R. DeBonte et al.

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Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-9. (Canceled)

10. (Previously Presented) The nucleic acid fragment of claim 66, wherein said at least one mutation comprises a codon encoding His in place of the codon encoding Leu.

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11-26. (Canceled)

27. (Previously Presented) An isolated nucleic acid comprising a full-length

Brassicaceae delta-15 fatty acid desaturase coding sequence having at least one mutation in a

region of said desaturase coding sequence encoding a His-Xaa-Xaa-Xaa-His amino acid motif,

wherein said at least one mutation renders the product of said desaturase coding sequence non-

functional and wherein said sequence includes said at least one mutation.

28. (Canceled)

29. (Previously Presented) The nucleic acid fragment of claim 27, wherein said

sequence encodes a microsomal gene product.

(Canceled)

31. (Previously Presented) The nucleic acid fragment of claim 27, wherein said at

least one mutation introduces a non-conservative amino acid substitution in said region.

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- 32. (Previously Presented) The nucleic acid fragment of claim 31, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).
- 33. (Previously Presented) The nucleic acid fragment of claim 32, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.
- 34. (Previously Presented) The nucleic acid fragment of claim 27, wherein said mutant desaturase coding sequence is from a *Brassica napus* plant.
- 35. (Previously Presented) A *Brassicaceae* plant containing a full-length coding sequence of a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is in a region encoding a His-Xaa-Xaa-His amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.
  - 36. (Canceled)
- 37. (Previously Presented) The plant of claim 35, wherein said mutation confers a decreased level of  $\alpha$ -linolenic acid in seeds of said plant.
- 38. (Original) The plant of claim 35, wherein said mutant desaturase gene encodes a microsomal gene product.
- 39. (Original) The plant of claim 35, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.
- 40. (Previously Presented) The plant of claim 39, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

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- 41. (Previously Presented) The plant of claim 40, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.
- 42. (Original) The plant of claim 35, wherein said mutant desaturase gene is from a Brassica napus plant.
  - 43. (Original) The plant of claim 35, wherein said plant is a Brassica napus plant.
  - 44. (Previously Presented) A Brassicaceae plant containing:
  - a) a full-length coding sequence from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one delta-12 gene mutation in a region encoding a His-Xaa-Xaa-His amino acid motif; and
  - b) a full-length coding sequence from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-His amino acid motif;

wherein said delta-12 gene mutation and said delta-15 gene mutation render the products of said delta-12 desaturase gene and said delta-15 desaturase gene, respectively, non-functional.

- 45. (Previously Presented) The plant of claim 44, wherein said mutant genes confer a decreased level of  $\alpha$ -linolenic acid in seeds of said plant compared to  $\alpha$ -linolenic acid levels in corresponding seeds lacking said mutant genes.
- 46. (Previously Presented) A Brassicaceae or Helianthus plant containing a full-length coding sequence of a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

47-54. (Canceled)

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- 55. (Currently amended) A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:
  - a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
  - b) obtaining one or more plants from said cells;
  - c) identifying at least one of said plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation in said delta-12 gene renders the product of said delta-12 desaturase gene non-functional; and
  - d) producing said <u>Brassicaceae or Helianthus</u> plant line from said at least one plant, said <u>Brassicaceae or Helianthus</u> plant line having said at least one mutation in said delta-12 gene.
- 56. (Original) The method of claim 55, wherein said plant line yields an oil having a stabilized linoleic acid content from about 2.0 % to about 12.0 %.
  - 57. (Currently amended) The method of claim 55, further comprising the steps of:
    - e) inducing mutagenesis in cells of said <u>Brassicaceae</u> or <u>Helianthus</u> plant line;
    - f) obtaining one or more plants from said cells of said <u>Brassicaceae or</u>

      Helianthus plant line;
    - g) identifying at least one of said plants from step f) that contains a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation in said delta-15 gene is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 desaturase gene non-functional; and

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- h) producing a second <u>Brassicaceae</u> or <u>Helianthus</u> plant line from said at least one plant identified in step g), said second plant line having said at least one mutation in said delta-12 gene and said at least one mutation in said delta-15 gene.
- 58. (Original) The method of claim 55, wherein said starting variety is a *Brassica* napus variety.
- 59. (Original) The method of claim 58, wherein said mutation is in a first form of delta-12 fatty acid desaturase.
- 60. (Original) The method of claim 59, further comprising the step of crossing a plant of said plant line to a plant having a mutation in a second form of delta-12 fatty acid desaturase.
- 61. (Original) The method of claim 60, wherein said second mutation is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.
  - 62. (Currently amended) The method of claim 59, further comprising the steps of:
  - e) inducing mutagenesis in cells of said <u>Brassicaceae</u> or <u>Helianthus</u> plant line;
  - f) obtaining one or more plants from said cells of said <u>Brassicaceae</u> or <u>Helianthus</u> plant line;
  - g) identifying at least one of said plants from step f) that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-His amino acid motif; and

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- h) producing a second <u>Brassicaceae</u> or <u>Helianthus</u> plant line from said at least one plant identified in step g), said second <u>Brassicaceae</u> or <u>Helianthus</u> plant line having said first and second delta-12 gene mutations.
- 63. (Original) The method of claim 55, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.
- 64. (Currently amended) A method for producing a *Brassicaceae* plant line, comprising the steps of:
  - a) inducing mutagenesis in cells of a starting variety of a Brassicaceae or Holianthus species;
  - b) obtaining one or more plants from said cells;
  - c) identifying at least one of said plants that contains a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said delta-15 desaturase gene non-functional; and
  - d) producing said <u>Brassicaceae</u> plant line from said at least one plant, said <u>Brassicaceae</u> plant line having said mutation in said delta-15 gene.
- 65. (Original) The method of claim 64, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

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mutation.

- 66. (Currently amended) An isolated nucleic acid comprising a [[a]] full length Brassicaceae or Helianthus delta-12 fatty acid desaturase coding sequence having at least one mutation in a region of said desaturase coding sequence encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif, wherein said at least one mutation renders the product of said desaturase coding sequence non-functional and wherein said sequence includes said at least one
- 67. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* plant, comprising:
  - a) providing a Brassicaceae plant having a decreased  $\alpha$ -linolenic acid content as compared with a corresponding control Brassicaceae plant; and
  - b) identifying at least one mutation in a delta-15 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 fatty acid desaturase gene non-functional.
- 68. (Previously Presented) The method of claim 67, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.
- 69. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* or *Helianthus* plant, comprising:
  - a) providing a Brassicaceae or Helianthus plant having an increased oleic acid content as compared with a corresponding control Brassicaceae or Helianthus plant; and
  - b) identifying at least one mutation in a delta-12 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His

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amino acid motif, wherein said mutation renders the product of said delta-12 fatty acid desaturase gene non-functional.

70. (Previously Presented) The method of claim 69, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.